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### REMARKS

This application is a Rule 53(b) continuation application of application no. 08/883,435, filed April 7, 1997, ("the parent application"), now issued as U.S. Patent 6,333,181. Claims 1-25 were pending in the application. Claims 1 and 7 have been amended and claim 26 has been added to claim more fully the instant invention. Accordingly, claims 1-26 will be pending upon entry of the instant Amendment.

Support for the amendments to claims 1 and 7 and for the addition of claim 26 can be found in the specification and claims as originally filed. In particular, support can be found in the specification at least, for example, in working Example 1 on pages 15-16 and in working Example 4 on pages 18-19. No new matter has been added. For the Examiner's convenience, the claims that will be pending upon entry of the claim amendments herein are set forth in Appendix A attached hereto.

Amendment of the claims is not to be construed as an acquiescence to any of the rejections set forth in the instant Office Action and was done solely to expedite prosecution of the application. Applicants reserve the right to prosecute the claims as originally filed in this or subsequent applications.

### *Specification*

The Office Action, at page 2, first paragraph, indicates that the preliminary amendment of the specification (item 5, page 2 of the submission of December 21, 2001) could not be entered because of an extraneous quotation mark within the body of the text to be added. Applicants apologize for the inadvertent typographical error that resulted in this extraneous quotation mark.

However, as the proposed text is clearly a standard addition to a Rule 53(b) continuation application, Applicants submit that what was intended to be added was nevertheless clear. Accordingly, page 1 of the specification has been amended to reflect the clear intent. Applicants also inserted the number of the patent that subsequently issued from the parent application.

The Office Action also indicates that the attempt to incorporate subject matter into the application by reference to applications referenced only by attorney docket numbers is improper. Because the two applications were filed concurrently with the parent application, the serial numbers were not available at the date of filing. Both applications, however, were clearly identified by the unique docket codes assigned to the applications and by their respective dates of filing. Accordingly, page 11 of the specification has been amended to insert the serial numbers of the two previously referenced US patent applications, as well as to add the corresponding numbers of the patents that have subsequently issued. Applicants submit that no new matter has been added to the application by the amendment to page 11.

### ***Claim Rejections – 35 U.S.C. §102***

#### **Rejection Of Claims 1-4 and 6 Under 35 U.S.C. §102(b) As Anticipated by Nakao *et al.***

Claims 1-4 and 6 are rejected under 35 U.S.C. §102(b) as being anticipated by Nakao *et al.*, *Ann. N.Y. Acad. Sci.*, 613 (Enzyme Engineering 10): 802-807 (1990) because according to the Examiner at page 2-3 of the Office Action:

Nakao discloses a process for increasing the extent of saccharification of lignocellulose, whereby lignocellulose is treated continuously with ultrasound as 20 kHz and a cellulase-containing microorganism, *Tricoderma viride*. Nakao therefore anticipates the cited claims.

Applicants respectfully disagree and traverse the rejection. However, without acquiescing to the rejection and in order to expedite prosecution, claim 1 has been amended so that the method is carried out "without pretreatment of said lignocellulose with alcohol/sodium oxide or alkaline pretreatment...".

Nakao *et al.* disclose alkaline pretreatments of the lignocellulose substrate followed by supersonic treatment in order to obtain saccharification. At page 807 of the reference, in conclusion (a), it is stated that "[f]or the ball-milled (*unpretreated*) lignocellulose, supersonic wave had an adverse effect". [Emphasis added.] In contrast to the process disclosed in Nakao *et al.*, the method of the invention as claimed herein does not require an alkaline pretreatment, as is demonstrated by Example 1 on pages 15-16 and in working Example 4 on pages 18-19 of the instant specification. Therefore, Applicants submit that claim 1, and claims 4-6 depending therefrom, are patentable over Nakao *et al.*, and respectfully request reconsideration and withdrawal of the rejection.

#### **Rejection Of Claims 1, 2 and 4 Under 35 U.S.C. §102(b) As Anticipated by Rolz**

Claims 1, 2 and 4 are rejected under 35 U.S.C. §102(b) as being anticipated by Rolz, *Biotech. Lett.*, 8(2):131-136 (1986)) because according to the Examiner at page 3 of the Office Action:

Rolz discloses a process for increasing the extent of saccharification of lignocellulose, whereby lignocellulose is treated continuously with ultrasound and cellulase. Nakao (sic) therefore anticipates the cited claims.

Applicants respectfully disagree and traverse the rejection. However, without acquiescing to the rejection and in order to expedite prosecution, claim 1 has been amended so that the method is carried out "without pretreatment of said lignocellulose with alcohol/sodium oxide or alkaline pretreatment...".

Like Nakao *et al.*, Rolz discloses alcohol/sodium oxide and alkaline pretreatments of the lignocellulose substrate followed by ultrasonic treatment to increase saccharification. At page 131 of the reference, Rolz concludes:

"In the present work we provide experimental evidence that an ultrasound field during enzymatic hydrolysis favors the rate of reaction *when the substrate surface has been pretreated.*" (Emphasis added.)

In contrast to the process disclosed in the Rolz reference, the method of the invention as claimed herein does not require an alcohol/sodium oxide or alkaline pretreatment. Therefore, Applicants submit that claim 1, and claims 2 and 4 depending therefrom, are patentable over the Rolz reference, and request reconsideration and withdrawal of the rejection.

### ***Claim Rejections – 35 U.S.C. §103***

#### **Rejection Of Claims 1, 2 and 5 Under 35 U.S.C. §103 In View of Nakao *et al.***

Claims 1, 2 and 5 are rejected under 35 U.S.C. §103 as being unpatentable over Nakao *et al.*, *Ann. N.Y. Acad. Sci.*, 613 (Enzyme Engineering 10):802-807 (1990).

According to the Examiner at page 4 of the Office Action:

...Nakao discloses the processes recited in claims 1 and 2. Nakao differs from the process recited in claim 5 in that Nakao does not apply the ultrasound discontinuously. However, the artisan of ordinary skill at the time of applicant's invention having before him the Nakao reference would have reasonably expected that the decision of whether to apply the ultrasonic irradiation continuously or discontinuously would have been a matter of design choice, either method being expected to function equivalently to the other.

Applicants respectfully disagree and traverse the rejection. However, without acquiescing to the rejection and in order to expedite prosecution, claim 1 has been amended to recite that "the lignocellulose has not been pretreated with an alcohol/sodium oxide or alkaline pretreatment...". As noted above, Nakao *et al.* disclose that an alkaline pretreatment step is required for ultrasound to increase effectively the rate of saccharification, stating at page 137 that "[f]or the ball-milled (unpretreated) lignocellulose, supersonic wave had an adverse effect". [Emphasis added.] Thus, the Nakao reference alone is insufficient to obviate claims 1, 2, and 5 because its disclosure constitutes a clear teaching away from the presently claimed invention which recites a process without a pretreatment step. Therefore, Applicants submit that claim 1, and claims 2 and 5 depending therefrom, are not obvious in view of Nakao *et al.*, and respectfully request reconsideration and withdrawal of the rejection.

**Rejection Of Claims 7-25 Under 35 U.S.C. §103 In View of Ingram *et al.* and Nakao *et al.***

Claims 7-25 were rejected under 35 U.S.C. 103(a) as being unpatentable over Ingram *et al.* (U.S. Pat. 5,424,202) in view of Nakao *et al.*, *Ann. N.Y. Acad. Sci.*, 613 (Enzyme Engineering 10):802807 (1990) and Rolz, *Biotech. Lett.* 8(2):131-136 (1986). Applicants respectfully disagree and traverse the rejection.

As admitted on page 5 of the Office Action, Ingram *et al.* do not disclose the use of ultrasound in the degradation of cellulose. At page 5, first full paragraph, the instant Office Action indicates that the "claims do not exclude any of the pretreatment steps in either Nakao or Rolz". Applicants submit that the claims at issue need not exclude these pretreatment steps as the claims are distinguishable over the processes disclosed in the references for other reasons. The method recited in claim 7 involves the use of an ethanologenic microorganism *in addition to a cellulase*. In contrast, the cited references disclose the use of a microorganism (*Trichoderma viride*) to provide the cellulase enzyme only. The cited references, alone or in combination, neither teach nor suggest the use of an ethanologenic microorganism to convert the cellulose, resulting from the cellulase-

catalyzed hydrolysis of lignocellulose, to ethanol, in addition to a cellulase. In view of the combination of the failure of Ingram *et al.* to teach or suggest the use of ultrasound in the degradation of cellulose with the failure of the Nakao and Rolz references to teach or suggest the use of an ethanologenic microorganism, Applicants submit that one of ordinary skill in the art would have no motivation to combine the references in the manner suggested in the instant Office Action.

In this regard, Applicants invite the Examiner's attention to the enclosed copies of: Scherba, G. *et al.*, "Quantitative Assessment of the Germicidal Efficacy of Ultrasonic Energy", *Appl. Environ. Microbiol.*, 57(7): 2079-2084 (July 1991); Tiehm, A. *et al.*, "The Use of Ultrasound to Accelerate the Anaerobic Digestion of Sewage Sludge", *War. Sci. Tech.*, 36(11): 121-127 (1997); and Earnshaw, R.G. *et al.*, "Understanding Physical Inactivation Processes: Combined Preservation Opportunities Using Heat, Ultrasound and Pressure", *Int. J. Food Microbiology*, 28:197-219 (1995). These papers are cited on the PTO 1449 accompanying an Information Disclosure Statement filed on even date herewith. These papers teach one of ordinary skill in the art that the application of ultrasound as taught in the instant invention would most likely kill the ethanologenic microorganism needed to convert the hydrolyzed cellulose to ethanol. See, for example the abstract and page 7, right column, first full paragraph of Scherba *et al.*; page 124, sentence just above Figure 4 of the Tiehm *et al.* reference; and Sections 3 and 3.1, pages 203-205 of the Earnshaw *et al.* reference. In view of the teachings of these papers, Applicants submit that one of ordinary skill in the art would have no motivation to combine the references in the manner suggested in the Office Action.

Indeed such motivation can only be provided by an application of the teachings of the instant invention, and such is improper as it constitutes a hindsight reconstruction of Applicants' invention. Moreover, assuming, *arguendo*, such a motivation existed, one of ordinary skill in the art still would have no expectation of success, as these three

references teach that application of ultrasound would significantly reduce if not block the activity of the ethanologenic microorganism.

Therefore, Applicants submit that claim 7, and claims 8-25 depending therefrom, are not obvious over Ingram *et al.* in view of Nakao *et al.* and Rolz, and respectfully request reconsideration and withdrawal of the rejection.


### ***Double Patenting***

Claims 1-25 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-16 of U.S. Patent 6,333,181. Applicants will address the double patenting rejection upon a finding by the Examiner that the claims are in condition for allowance but for the double patenting rejection.

### **CONCLUSION**

Applicants respectfully request entry of the foregoing amendments and remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application with claims 1-26 is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Respectfully submitted,  
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Date: November 26, 2003



## APPENDIX A

1. A method for enzymatically degrading lignocellulose comprising the steps of:
  - (a) treating an aqueous mixture containing lignocellulose, without pretreatment of said lignocellulose with an alcohol/sodium oxide or alkaline pretreatment, with ultrasound; and
  - (b) contacting the mixture with a cellulase under conditions sufficient for hydrolysis.
2. The method according to Claim 1 wherein said aqueous mixture of step (a) further comprises said cellulase.
3. The method according to Claim 2 wherein said cellulase is provided by a cellulase-producing microorganism in said aqueous mixture.
4. The method according to Claim 2 wherein said step (a) is continuous.
5. The method according to Claim 2 wherein said step (a) is discontinuous.
6. The method according to Claim 1 wherein said ultrasound is conducted at a frequency of between about 2 and 200 kHz.
7. A method for enzymatically degrading lignocellulose comprising the steps of:
  - (a) treating an aqueous mixture containing lignocellulose with ultrasound; and
  - (b) contacting the mixture with a cellulase and ethanologenic microorganism under conditions sufficient for hydrolysis.

8. The method according to Claim 7 wherein said aqueous mixture of step (a) further comprises said cellulase and ethanologenic microorganism.

9. The method according to Claim 8 wherein said cellulase is provided by a cellulase-producing microorganism in said aqueous mixture.

10. The method according to Claim 8 wherein said step (a) is continuous.

11. The method according to Claim 8 wherein said step (a) is discontinuous.

12. The method according to Claim 8 wherein said ultrasound is conducted at a frequency of between about 2 and 200 kHz.

13. The method according to Claim 8 wherein said ethanologenic microorganism is an ethanologenic bacteria or yeast.

14. The method according to Claim 13 wherein said ethanologenic microorganism is a bacteria or yeast which expresses one or more enzymes which, individually or together, convert a sugar to ethanol.

15. The method according to Claim 13 wherein said ethanologenic microorganism expresses enzymes which, individually or together, convert pentose and hexose to ethanol.

16. The method according to Claim 13 wherein said ethanologenic microorganism expresses alcohol dehydrogenase and pyruvate decarboxylase.

17. The method according to Claim 16 wherein said alcohol dehydrogenase and pyruvate decarboxylase are from *Zymomonas mobilis*.

18. The method according to Claim 13 wherein said ethanologenic microorganism expresses xylose isomerase, xylulokinase, transaldolase, and transketolase.

19. The method according to Claim 18 wherein said xylose isomerase, xylulokinase, transaldolase, and transketolase are from *Escherichia coli*.

20. The method according to Claim 18 wherein said xylose isomerase, xylulokinase, transaldolase, and transketolase are from *Klebsiella oxytoca*.

21. The method according to Claim 18 wherein said xylose isomerase, xylulokinase, transaldolase, and transketolase are from *Erwinia* species.

22. The method according to Claim 13 wherein said ethanologenic microorganism expresses alcohol dehydrogenase, pyruvate decarboxylase, xylose isomerase, xylulokinase, transaldolase, and transketolase.

23. The method according to Claim 22 wherein said ethanologenic microorganism is a recombinant microorganism expressing *Zymomonas mobilis* alcohol dehydrogenase and pyruvate decarboxylase wherein said microorganism is selected from the group consisting of *Escherichia coli*, *Klebsiella oxytoca*, and *Erwinia* species.

24. The method according to Claim 23 wherein said ethanologenic microorganism is *Klebsiella oxytoca* P2.

25. The method according to Claim 23, wherein said ethanologenic microorganism is *Escherichia coli* KO11.

26. The method of claim 7, wherein step (a) is performed without pretreatment of said lignocellulose with an alcohol/sodium oxide or alkaline pretreatment.